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Fibonacci Primes identifying Fibonacci Primes Hexagon and Fibonacci Primes Spider. The center of the spider consists of Fibonacci primes 17 and 19 which move in the direction of increasing Fg numbers as congruence (mod 3), and the spider enlarges in a systematic pattern. Fa and Fg sequences determine specific positions in applications of Fibonacci primes to the two asymmetric halves of tRNA, mRNA, as well as protein biomolecules. [1] L.E. Sigler, *Fibonacci's Liber Abaci: A Translation in Modern English of Leonardo Pisano's Book of Calculations*, New York, Springer-Verlag, 2002.

DNA Replication, Recombination & Repair

1309-Pos Board B219

Single-Molecule Studies of the Eukaryotic Replicative DNA Helicase MCM2-7

Hasan Yardimci, Antoine M. van Oijen, Johannes C. Walter.

In eukaryotes, double hexamers of the replicative DNA helicase MCM2-7 are loaded onto double-stranded DNA (dsDNA) at each origin of replication in the G1 phase of the cell cycle. In S phase, numerous accessory factors activate the helicase activity of MCM2-7, leading to unwinding of the DNA template. Despite decades of study, it remains unclear how MCM2-7 unwinds DNA. One model is that upon activation, MCM2-7 goes through a conformational change to encircle single-stranded DNA (ssDNA) whereupon it translocates along one strand while excluding the other (steric exclusion). An alternative model envisions that MCM2-7 translocates along dsDNA. As DNA emerges the rear exit channel of MCM2-7, it is split by a rigid pin that bisects the channel. To distinguish between the two scenarios, we used an experimental system that allows single-molecule visualization of DNA replication in *Xenopus* egg extracts. We attached a quantum-dot (Qdot) to lambda DNA at a specific location that served as a road block for replication forks. Upon exposure of such Qdot-labeled lambda DNA to extracts in a microfluidic flow cell, a significant fraction of forks bypassed the Qdot when it was located on the lagging strand template but not when it was located on the leading strand template. Our results support the model that MCM2-7 translocates in the 3' to 5' direction along ssDNA and unwinds DNA by sterically excluding the opposite strand. Our results suggest that the large number of factors that are required to activate the MCM2-7 complex at the G1/S transition function by remodeling the MCM2-7 complex from a dsDNA binding mode to a ssDNA binding mode.

1310-Pos Board B220

Dna Unwinding Dynamics of a Processive DNA Polymerase

Borja Ibarra, Jose Morin, Francisco Cao, Margarita Salas, Jose M. Valpuesta, Jose L. Carrascosa.

During DNA replication mechanical unwinding of the DNA helix is required for the advance of the replication machinery. Unlike many DNA polymerases the bacteriophage Phi29 DNA polymerase presents a processive 'helicase-like' activity and is able to couple DNA replication and unwinding within the same polypeptide. Using Optical Tweezers we have developed a single molecule mechanical assay to elucidate the physical mechanism of DNA unwinding by the Phi29 DNA polymerase as the protein replicates processively the DNA. A DNA hairpin is held between an optical trap and a mobile surface. As a single polymerase works on the hairpin its replication and unwinding activities can be measured in real time (by measuring the change in extension in the DNA polymer), revealing the fluctuations of their rates in response to the DNA sequence and force applied in the direction of unwinding. The sequence and force sensitivities of the unwinding reaction of the wild type and an unwinding-deficient polymerase mutant indicate that the Phi29 DNA polymerase presents an active unwinding mechanism that may substantially differ from the unwinding mechanism used by specialized nucleic acid helicases.

1311-Pos Board B221

Recombination Hotspots and SSB Proteins Couple Translocation and Unwinding Activities of the AddAB Helicase-Nuclease

Joseph T.P. Yeeles, Kara van Aelst, Mark S. Dillingham, **Fernando Moreno-Herrero**.

Recombinational repair of DNA breaks requires processing of a DNA end to a 3'-ssDNA overhang. In *B. subtilis*, this task is done by the helicase-nuclease AddAB which generates ssDNA overhangs terminated at a recombination hotspot (Chi) sequence. In this work, we have used stopped flow DNA unwinding assays and atomic force microscopy to investigate the processing of DNA breaks by the AddAB helicase-nuclease. In the absence of single-stranded binding proteins, we found that translocation and unwinding activities of AddAB

are uncoupled due to re-annealing of nascent single-stranded DNA. However, recognition of Chi sequences during AddAB translocation activates unwinding by coupling both activities. Helicase activity of AddAB is also activated by binding of SSB proteins or activity of multiple AddAB in multiple turnover reactions by preventing re-annealing of DNA strands. The implications of these findings for our understanding of DNA break repair intermediates and of general helicase mechanisms will be discussed.

1312-Pos Board B222

Segregation of Sister Chromosomes in *E. coli* is Governed by the Shape of the Nucleoid and the Release of Inter-Sister "snaps"

Jay K. Fisher, Aude Bourniquel, Mara Prentiss, Nancy Kleckner.

E. coli chromosome dynamics occurring throughout the cell cycle, as defined by fluorescence microscopy of living cells grown in a microfluidic device, have been analyzed in 3D at high resolution. Imaging of the nucleoid (HUMCherry) reveals an asymmetric left-handed helicoidal ellipsoid. The shape can be seen to exert radial pushing forces at points of contact with the cell periphery, thereby defining complementary intracellular compartments. These compartments constrain the location of DNA replication and determine the paths taken by newly replicated DNA. As replication proceeds, two types of abrupt changes are observed: (i) rotation and modulation of the helix without change in basic shape; and (ii) longitudinal protrusion, first of one sister nucleoid towards the old cell pole and then of the other sister nucleoid towards the new cell pole. These two protrusion steps correspond to previously-described transitions involving release of specialized inter-sister "snaps". We propose that loss of the inter-sister "snaps" and ensuing nucleoid movements, as well as other dynamic effects seen later in the cell cycle, are driven by accumulation and release of mechanical stress arising from the intra-nucleoid repulsive forces, likely arising via interactions between negatively supercoiled plectonemes. We further propose that nucleoid shape arises via repulsion under confinement. Overall these findings lead to a picture in which the *E. coli* nucleoid is a "bag of springs", not a "bag of string", and where intrinsic physical properties of the DNA dictate the behaviors and dispositions of other components via mechanically-driven effects.

1313-Pos Board B223

Mechanism of Yeast Clamp Loading on DNA

Tae-Hee Lee, Stephen Benkovic, Padmaja Mishra, Ravindra Kumar.

Based on biochemical and single molecule fluorescence measurements using the yeast proliferating cell antigen (PCNA) and replication factor C (RFC), we studied the assembly of the RFC·PCNA·DNA complex and its progression to holoenzyme in the presence of polymerase δ (Pol δ). Our data indicate that i) PCNA loads to DNA through multiple conformational states; ii) PCNA loading is successful after several failed attempts; iii) there are two different states of PCNA loaded on DNA; iv) in the presence of Pol δ only one of the two states proceeds to the RFC·PCNA·DNA·Pol δ holoenzyme. These findings redefine and deepen our understanding of the clamp loading process and reveal that it is surprisingly one of trial and error to arrive at a heuristic solution.

1314-Pos Board B224

Nucleotide and DNA-Induced Structural Transitions and the Coupling Between ATP and DNA Binding Sites in RecA

Tao Jiang.

RecA is the prototype of ATPase proteins that mediates homologous DNA recombination. RecA requires ATP to promote the binding of DNA, as both ADP- and nucleotide-free states show low DNA binding affinity. We investigated how ATP binding affects the dynamics of DNA binding loops, and activates RecA by the interactions between β - γ -phosphate and Walker A and Walker B motifs, as well as through the contacts made between the C-terminal and the central domains within the protein. DNA binding results in the formation of the extended, active conformation of the RecA filament, which catalyzes strand exchange. We have performed a set of molecular dynamics simulations on the active RecA, with ATP/ADP/nucleotide-free bound, to investigate the conformational transitions between the active and inactive states. Our simulations have revealed that the structural changes upon ATP binding are confined to small motifs, while the conformational changes upon DNA binding involve larger scale rearrangement of the protein, namely the rotation of monomers with respect to each other. The results suggest that ATP binding stabilizes the L1 and L2 DNA binding loops, mediated through specific residues located between the ATP and DNA binding sites that sense the presence of γ -phosphate. Furthermore, DNA binding leads to monomer rotations to form the extended conformation by affecting the interfaces of the adjacent monomers with